Vascular Obstruction in Sickle Cell Disease

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Abstract

Initially, the pathophysiology of sickle cell anemia was attributed to deoxygenation-induced polymerization of a mutant form of hemoglobin, hemoglobin S, as well as sickling of red blood cells that plug blood vessels causing the onset of a painful vasoocclusive crisis. Although hemoglobin S polymerization is central to the pathophysiology of this disease, the initiation of a vasoocclusive episode may involve multiple factors that disrupt the steady state and promote intravascular red blood cell sickling. Reversible sickling, red blood cell abnormalities, and transient ischemic events in this disease may cause endothelial injury resulting in abnormal erythrocyte-endothelium interactions, induction of inflammatory cytokines, leukocyte recruitment, and altered vascular tone. These factors can promote intravascular sickling by delaying red blood cell transit times in the microcirculation. This review briefly describes the role of these factors and some novel therapeutic approaches based on anti-adhesive and anti-inflammatory agents.

Introduction

Sickle cell disease (SCD) involves a single point mutation in the hemoglobin (Hb) A gene that results in the substitution of valine for glutamic acid at the sixth position of the Hb \( \beta \) chain. The single point mutation results in the polymerization of this mutant HbA molecule, HbS (\( \beta^S \)-globin), and morphological deformation of red blood cells (RBCs) under deoxygenated conditions. The morbidity of SCD is due to recurring episodes of painful vasoocclusive crisis and multiple organ damage. The classical view of SCD has emphasized the blockage of blood vessels by sickled erythrocytes, known as SS cells that precipitate vasoocclusion. In fact, increased RBC rigidity is a major determinant of the flow abnormalities, hemolysis, and reduced RBC-life span (Kaul et al., 1996).

The painful vasoocclusive crisis that characterizes SCD could not propagate without SS cells, but initial events may involve a complex interplay of factors. The sickling tendency of SS cells is not sufficient to explain the great variability in clinical presentation. Multiple factors are likely to participate in the initiation and progression of a vasoocclusive crisis. Factors that may potentially affect RBC transit times in the microcirculation and contribute to vasoocclusion are increased RBC rigidity, abnormal SS cell adhesion to vascular endothelium, induction of inflammatory cytokines, increased leukocyte-endothelium interactions, and altered vascular tone. A better understanding of RBC factors and vascular pathobiology of SCD is essential to devise effective therapeutic approaches. This review will briefly describe the role of these factors in vascular obstruction and highlight some recent developments that have enhanced our understanding in this area.

Reversible Sickling In Vivo

In the steady state, SS cells constantly undergo reversible sickling in the circulation. Most SS cells are likely to sickle at the low oxygen tension (~40 mm Hg) encountered in the veins while unsickling at high oxygen tension (~160 mm Hg) encountered in the lungs. However, reversible sickling in vivo does not generally result in vasoocclusion, because the lag time for HbS polymerization is generally longer than the RBC capillary transit time. Therefore, most SS cells escape sickling during capillary transit (Eaton and Hofrichter, 1987). A prolonged transit time of RBCs in the terminal arterioles, capillaries, or immediate postcapillary venules would be conducive to polymerization of HbS and vasoocclusion. HbS polymerization also results in increased rigidity (i.e., abnormal rheology) of SS cells, which would deprive the RBCs of their ability to traverse narrow capillaries. The factors that promote sickling would play a critical role in the initiation of a vasoocclusive episode.

Reversible sickling does generates myriad abnormalities. Intravascular sickling and hemolysis will result not only in erythropoietic stress and increased reticulocyte production, but also in RBC heterogeneity, RBC membrane abnormalities, RBC-endothelium interaction, and endothelial damage. Blood in SCD patients contains RBCs of heterogenous densities characterized by differences in the mean corpuscular hemoglobin concentration (MCHC) (Fabry and Nagel, 1982). HbS polymerization rates are extremely sensitive to MCHC. Dense SS cells with high MCHC polymerize rapidly. The density-defined SS cell classes exhibit differences not only in their rheologic and hemodynamic properties but also in their adhesive and obstructive behavior (Kaul et al., 1983). The generation of dense SS cells is mainly attributed to cell dehydration secondary to abnormal transport properties of the RBC membrane (Brugnara, 1993). The increased RBC rigidity and erythrocyte-endothelium interactions may constantly inflict injury to the vascular endothelial lining and affect endothelial function as well as vascular reactivity. These abnormalities would contribute to vasoocclusive crisis and multiple organ damage.

SS Cell-endothelium Interactions

SS cells adhesion to the vascular endothelium is a major factor that could potentially trigger a vasoocclusive episode. Experimental evidence dating back to the sem-
Figure 1: The sequential model for SCD vasoocclusion. Preferential adhesion of light- and normal-density SS cells in postcapillary venules is followed by reduction in local wall shear rates and selective trapping of dense SS cells. This could result in HbS polymerization in the trapped and adhered SS cells as well as obstruction of the affected vessels (Based on Kaul et al., 1989).

Figure 2: Schematic representation of adhesion molecules involved in SS cell endothelium interactions (EC=endothelial cell, IAP=integrin-associated protein, Sulf. glycolipids=sulfated glycolipids).
inal observations of Hebbel et al. (1980), shows that SS cells adhere abnormally to vascular endothelium in a variety of assay systems involving both static and dynamic flow conditions (Kaul, 1994). Recent studies have shown that postcapillary venules are the exclusive sites of SS cell-endothelium interactions both in ex vivo and in vivo models (Kaul et al., 1989a; Kaul et al., 1995). Furthermore, heterogeneous SS cells contribute differentially to adhesion and vasoocclusion. Adhesion-induced vasoocclusion is a two-step process (Figure 1). Initial adhesion of deformable, light density SS cells (i.e., reticulocytes and discocytes) in postcapillary venules followed by selective trapping of dense SS cells and vessel blockage (Kaul et al., 1989b). The secondary trapping of dense SS cells in postcapillary venules would be followed by rapid HbS polymerization and sickling of these cells due to their high MCHC and may obstruct the whole feeding capillary network in a retrograde fashion. Thus, SS cell adhesion would result in a drastic reduction in the effective vessel-lumen diameter and local flow rates contributing to longer RBC transit times, trapping of dense SS cells, sickling, and vasoocclusion.

In SCD, abnormal RBC adhesion to vascular endothelium is probably the consequence of expression of adhesion molecules on both the SS cell and the vascular endothelium secondary to reversible sickling and endothelial cell damage. While repeated RBC sickling would result in membrane abnormalities and exposure of membrane components that may mediate SS cell adhesion, increased RBC destruction would result in excessive production of reticulocytes, particularly sticky stress reticulocytes. Further, intravascular sickling and transient ischemic episodes would result in endothelial cell damage and upregulation of adhesive molecules. Extensive work on human SS cell adhesion has revealed an array of adhesion molecules (Figure 2) that fall into three categories:

(a) **RBC receptors:** Receptors to adhesive proteins are mainly confined to stress reticulocytes that include the integrin receptor 41 and glycoprotein IV (CD36 or GPIV) (Sugihara et al., 1992; Brittain et al., 1993). Repeated sickling may also result in abnormally exposed membrane components (Band-3 and sulfated glycolipids) that may mediate adhesion of non-reticulocytes (Hillery et al., 1996; Thevenin et al., 1997). In addition, B-CAM/basal (Luther cell adhesion molecule presenting Lutheran blood group antigen) and integrin-associated protein (IAP) may also participate as RBC receptors (Udani et al., 1998; Brittain et al., 1999).

(b) **Adhesive bridging proteins:** These include thrombospondin (TSP), unusually large molecular weight forms of von Willebrand factor (vWF), and laminin. TSP binds to CD36, while vascular cell adhesion molecule (VCAM-1) expressed on cytokine-activated endothelium can bind α4β1 integrin (Figure 1). TSP, vWF, and laminin are known to bind sulfated glycolipids in vitro, and this binding is competitively inhibited by anionic polysaccharides. In addition, TSP and laminin could interact with IAP and B-CAM/Luth receptors on SS cells, respectively. Recent studies have shown that TSP-mediated SS cell adhesion to the endothelium is inhibited in the presence of certain anionic polysaccharides (large molecular weight dextran sulfate, chondroitin sulfate A, and heparin) (Barabino et al., 1999). These observations are in agreement with those obtained using immobilized TSP (Hillery et al., 1996; Joneckis et al., 1996). Subsequent studies by Hebbel et al. (1980) showed that TSP binding to SS cells was significantly inhibited in the presence of heparin, heparin sulfate, and chondroitin sulfate A (Gupta et al., 1999). Hebbel et al. (1980) has emphasized that the N-terminal domain of TSP is involved in mediating TSP-binding to endothelium via its interaction with heparin sulfate proteoglycan the role for endothelial heparin sulfate proteoglycan in TSP binding needs to be confirmed under flow conditions. TSP also interacts with endothelial αVβ3 via its RGD (arginine-glycine-aspartic acid) sequence. In addition, as shown by Hillery et al. (1999), the C-terminal cell-binding domain of TSP may have a crucial role in adhesion. This domain modulates αVβ3 function (Gao and Frazier, 1994) and binds to SS cells in immobilized form (Hillery et al., 1999). However, potential conformational changes in the proteins under immobilized forms may not necessarily reflect the situation in vivo.

(c) **Endothelial receptors:** Recent ex vivo studies have shown that the stimulation of artificially perfused mesocccum vasculature by platelet-activating factor promotes SS cell adhesion to postcapillary endothelium. This adhesive interaction is blocked by two monoclonal antibodies (7E3 and LM609) directed against endothelial αvβ3 integrin, demonstrating that αvβ3 is an important endothelial receptor in SS cell adhesion (Kaul et al., 2000a). Likewise, TSP-enhanced adhesion to the cultured endothelium is inhibited by anti-αvβ3 antibodies (Sugihara et al., 1992). Both vWF and TSP can bind to αvβ3 integrin receptors (Felding-Habermann and Cheresh, 1993; Gupta et al., 1999), and laminin is also reported to bind to αvβ3 and β1 receptors (Kramer et al., 1990). These adhesive proteins can also bind to sulfated glycolipids. Thus, tripartite adhesive complexes RBC receptor-adhesion protein-endothelial receptor may contribute to SS cell adhesion. Based on known interactions between these components, a number of such complexes may exist, including CD36-TSP-αvβ3 integrin, sulfated glycolipid-TSP-αvβ3, sulfated glycolipid-vWF-αvβ3, and sulfated glycolipid-laminin-αvβ3 integrin or several potential β1 integrins. Hence, αvβ3 integrin may play an important role in SS cell adhesion to vascular endothelium. The blockade of αvβ3 is a novel therapeutic approach to prevent SS cell-endothelium interactions and adhesion-induced vasoocclusive events.
Reperfusion Injury and Leukocyte-endothelium Interactions

In SCD, at least two factors would contribute to vascular pathology—reversible sickling and RBC adhesion to the vascular endothelium. Both these factors can cause endothelial damage and contribute to ischemic episodes. Furthermore, the initiation, progression, and resolution of a vasoocclusive crisis may present features common with “reperfusion injury.” Reperfusion injury is attributable to the reintroduction of molecular oxygen and consequent generation of oxidants that occurs after an ischemic episode (Grisham et al., 1998; Granger, 1999). Subclinical vasoocclusive events (Figure 3) involving a transient blockage of vascular bed by rheologically abnormal RBCs, intravascular sickling, and RBC adhesion are likely to be much more prevalent than the painful vasoocclusive episodes (Kaul and Hebbel, 2000). Repeated and random occurrence of these subclinical ischemic episodes will adversely affect vascular endothelial function and contribute to multiple organ damage. Such episodes of reperfusion injury would result in a proinflammatory state in SCD.

Reperfusion injury is characterized by leukocyte recruitment resulting in tissue dysfunction for various organ systems, including the heart, skeletal muscle, lungs, intestine, and skin (Granger, 1999). In SCD, increased leukocyte-endothelium interactions subsequent to reperfusion injury would significantly affect the microvascular transit times of SS cells. Thus, increased leukocyte recruitment in the microcirculation would enhance HbS polymerization, SS cell sickling, and vessel occlusion. This concept is supported by the following observations:

(a) Oxidant generation and leukocyte recruitment: There is ample support for the concept that reperfusion (i.e., resupply of oxygen) can paradoxically injure ischemic tissues. This is referred to as the “oxygen paradox,” in which much of the injury occurs during the period when molecular oxygen is reintroduced into the tissue after ischemia (Granger et al., 1981). Briefly, ischemia results in depletion of ATP, accumulation of its metabolites such as hypoxanthine, and the conversion of the enzyme xanthine dehydrogenase to xanthine oxidase (Grisham et al., 1998). Reperfusion allows xanthene oxidase to act on its purine substrate and generate oxidants. The endothelium is an early and prominent target of reperfusion injury as it is relatively rich in xanthine oxidase although reperfusion injury is not limited to endothelial cells. Adherence and activation of neutrophils are generally considered as crucial to reperfusion injury. In addition to the generation of hydrogen peroxide ($H_2O_2$), highly toxic hydroxyl radicals (OH) may form in the presence of ferrous iron (Hebbel et al., 1982). Alternative mechanisms that can generate OH radicals include the interaction of free radical nitric oxide (NO) with superoxide to form peroxynitrite (ONOO$^*$), which can decompose at physiological pH to generate OH

(Crow and Beckman, 1995).
Leukocyte-endothelium interaction involves initial rolling (i.e., repeated transient contacts) of leukocytes along the endothelial surface followed by their firm adhesion and diapedesis. The rolling is mediated by selectins expressed on activated endothelial cells (McEver, 1994; Carlos and Harlan, 1994). Upon initial activation of endothelium by oxidants, histamine, and thrombin, P-selectin is translocated to the endothelial surface within minutes. P-selectin supports rapid induction of leukocyte rolling in postcapillary venules. On the other hand, expression of E-selectin requires biosynthesis and is inducible by cytokines with maximal surface expression four to six hours after stimulation. Leukocyte rolling on the activated endothelium allows activation of two integrins (CD11/CD18) expressed on leukocytes (Carlos and Harlan, 1994). The interaction between CD11/CD18 integrins (e.g., CD11a and CD11b) with endothelial ligands, such as intracellular adhesion molecule (ICAM-1), results in arrest and adhesion of leukocytes on endothelial surface. Because leukocytes are more rigid and have a larger volume than RBC, an increase in their number and their enhanced interaction with endothelium would adversely affect overall microvascular hemodynamics and vascular resistance.

Figure 4: Evidence of oxidant generation in vascular endothelial cells in transgenic SCD mice. Oxidant production was monitored by the use of dihydrorhodamine 123 (DHR). In the presence of \( \text{H}_2\text{O}_2 \), DHR is converted to the fluorescent compound, rhodamine. Representative fluorographs of cremaster venules in transgenic SCD mice (top) after DHR application and their corresponding fluorescence profile analysis (bottom). A) Normoxic control shows minimal oxidant activity. B) After 3 hours of hypoxia and 30 minutes of reoxygenation, oxidant generation was evident by intense fluorescence in the vascular endothelial cells. Bar = 10 \( \mu \text{m} \) (Modified from Kaul and Hebbel, 2000).

(b) Evidence of reperfusion injury in SCD: Intravascular sickling, RBC adhesion, and transient vasoocclusive events are likely to result in the reported endothelial injury in SCD patients (Klug et al., 1982). Another manifestation of vascular injury in SCD is the presence of sloughed-off endothelial cells in the peripheral circulation (Sowemimo-Coker et al., 1989; Solovey et al., 1997). Similarly, detachment of endothelial cells from the basement membrane is known to occur following reperfusion injury (Conger and Weil, 1995). Studies by Hebbel et al. (1980) have demonstrated that circulating endothelial cells in SCD patients have an activated phenotype (Solovey et al., 1998a; Solovey et al., 1998b). Consistent with reperfusion injury, there is evidence of oxidative damage (i.e., peroxynitrite formation) in SCD (Rybicki, 1999) implying an increased oxygen radical generation. A proinflammatory condition in SCD is indicated by an increase in the peripheral leukocyte counts (Buchanan and Glader, 1978; Boggs et al., 1973), elevated cytokines (Francis and Haywood, 1992), and an increase in soluble ICAM-1 and VCAM-1 in the plasma (Stuart and Setty, 1999). Another potent inflammatory agent, platelet-activating factor, is elevated in SCD patients (Oh et al., 1997). In SCD patients, infections are often followed by the occurrence
of a painful vasoocclusive crisis (Barrett-Connor, 1971). More significantly, base-line leukocyte counts are implicated as a major risk factor for severity in this disease (Platt, 2000). Based on these observations and consistent with the involvement of leukocytes in reperfusion injury, one would expect a crucial role for increased leukocyte-endothelial interactions in the pathophysiology of SS cell vasoocclusion.

(c) In vivo studies with transgenic SCD mice: To test the hypothesis that increased in vivo sickling followed by reoxygenation would trigger an inflammatory response, the effect of hypoxia/reoxygenation were investigated in transgenic SCD mice expressing approximately 75% human β-S-globin. In these mice, even at ambient air, peripheral leukocyte counts are elevated by 1.7-fold and neutrophil counts by almost 3-fold, consistent with observation in human SS patients. SCD mice, but not normal mice, showed a distinct inflammatory response characterized by an increased number of adherent and emigrated leukocytes when exposed to three hours of hypoxia was followed by reoxygenation. Because these events, which are exaggerated in SCD mice, are not seen in response to hypoxia alone, it was concluded that they represent a form of reperfusion injury. Oxidant production was monitored by using dihydorhodamine 123 (DHR), an H$_2$O$_2$ -sensitive probe. The results revealed a clear evidence of DHR oxidation in vascular endothelial cells after hypoxia/reoxygenation in the SCD mice (Figure 4). No such response was observed in control mice. These studies extend the finding of Osarogiagbon et al. (2000) that show both lipid peroxidation and OH radical generation occur in the same SCD mouse model under the same experimental conditions. Finally, in the SCD mice, infusion of an anti-P-selectin antibody, which inhibits initial leukocyte rolling contact with endothelium, caused a complete inhibition of the inflammatory response and a significant increase in microvascular flow velocities suggesting the therapeutic potential of anti-selectin therapy.

These novel findings indicate that hypoxia-induced sickling is involved in the inflammatory response to reoxygenation in the SCD mice. Oxidant generation and leukocyte recruitment characterize this response. Furthermore, these findings suggest that leukocyte-endothelium interactions will contribute to vasoocclusive events in the SCD mice and perhaps in human SCD.

Vascular Tone

As noted before, there is a strong evidence of endothelial damage, and the presence of sloughed-off endothelial cells in the circulation of SCD patients (Klug et al., 1982; Sovemi-mo-Coker et al., 1989; Solovey et al., 1997). This is similar to the situation reported for other ischemic diseases (Conger and Weil, 1995). One consequence of endothelial dysfunction would be altered vascular tone and hemodynamic adjustments, as suggested by reports of lower peripheral resistance and cardiovascular adjustments in SCD patients (Lonsdorfer et al., 1983; Lipowsky et al., 1987; Rodgers et al., 1984). Patients with SCD show an oscillatory flow pattern (Rodgers et al., 1984) and depressed vascular tone response to hyperemia (Lipowsky et al., 1987).

Transgenic SCD mouse models provide further insights into this aspect. In vivo studies in a transgenic mouse model (expressing human β$^S$- and β$^S$-Antilles-globins) show an attenuated vasoconstriction response to oxygen (Kaul et al., 1995), which is consistent with a role of NO and/or prostaglandins in modulating this behavior (Messina et al., 1994; Pries et al., 1995). This is accompanied by increased endothelial nitric oxide synthase (eNOS) expression in this SCD mouse model (Kaul et al., 2000b). Further, the increased eNOS/NO activity is associated with a lower blood pressure and a diminished arteriolar response to NO-mediated vasodilators, such as acetylcholine and sodium nitroprusside but a normal response to cyclic AMP-mediated vasodilators, such as forskolin.

Studies with the more severe transgenic-knockout Berkeley mouse (expressing human α$^S$- and β$^S$-globins exclusively) provide a strong evidence of a depressed vascular tone (Kaul et al., 1999). This is exemplified by a greater reduction of blood pressure, pronounced vasodilation, and an impaired NO-mediated vasorelaxation. In addition, blunted arteriolar constrictions in response to hyperoxia as well as to L-NAME, a NOS inhibitor, reflect a depressed vascular tone. These observations also suggest induction of alternative vasodilatory mechanisms and/or a deficit in vascular tone due to a loss of response to vasoconstrictors. Consistent with the view that SCD is a state of inflammation (Osarogiagbon et al., 2000; Kaul and Hebbel, 2000) generation of reactive oxygen species (Hebbel et al., 1982) in this disease could potentially induce certain vasodilator systems such as cyclooxygenase-2 and heme-oxygenase-1. In transgenic SCD mice, impaired NO-mediated vasodilation is accompanied by increased lipid peroxidation, suggesting a role for oxidants (e.g., H$_2$O$_2$) in an altered vascular tone. Although an increased vasodilator activity may compensate for flow abnormalities, it may also cause pathophysiological alterations and destabilization of vascular tone that may contribute to hemodynamic abnormalities and vasoocclusive events.

Conclusions

This review has presented an overview of the major factors that would probably contribute to vasoocclusive crisis in SCD. Clinical and experimental evidence indicates that sickling is essential but may not be sufficient for vasoocclusion, and multiple factors may participate in disruption of the steady-state RBC morphology leading to sickling and vasoocclusion. Thus, the factors that promote sickling by prolonging RBC transit times in the microcirculation would have an important role in the initiation of vasoocclusion. Reversible sickling in vivo would generate various
abnormalities involving both RBCs and vascular endothelium. This is evident by abnormal adhesion of SS cells to vascular endothelium. Furthermore, reperfusion injury and oxidant generation secondary to ischemia/reoxygenation would trigger increased leukocyte-endothelium interactions. Both these factors would facilitate SS cell sickling by prolonging the RBC transit times. Therapeutic strategies to inhibit SS cell interaction with endothelium offer a potential approach to the treatment of the disease. An example of this approach is blockade of endothelial αvβ3 integrin receptor by monoclonal antibodies. In view of the potential role of reperfusion injury in this disease, therapies designed to interfere with leukocyte adhesion mechanisms (e.g., anti-P-selectin antibody) and/or with oxidant generation constitute another promising approach. In addition, upregulation of vasoactive molecules (e.g., NO, cyclooxygenase-2, and heme-oxygenase-1) secondary to ischemic insult and reperfusion injury may induce vascular tone modifications affecting systemic blood pressure and microvascular flow dynamics. Altered vascular tone responses to vasodilators and constricting stimuli may adversely affect normal vasomotion and the ability of microvasculature to respond to the rheological challenge. A better understanding of these factors could potentially lead to novel therapeutic strategies in the treatment of SCD.

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References


Rybicki, A. C. Accumulation of nitrotyrosine in the light and dense fractions of sickle red blood cells. National sickle cell disease program, 23rd annual meeting, San Francisco, March 6-9, 1999.


